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Antimicrobial Flavonoid Rutin from *Pteris vittata* L. against Pathogenic Gastrointestinal Microflora

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ABSTRACT.—*Pteris vittata*, commonly known as 'Brake Fern', is a relatively uninvestigated species as far as antimicrobial activity is concerned. Different aqueous methanolic (70, 80, and 90%) extracts of *P. vittata* were tested for the growth of eight intestinal microorganisms, by using disc diffusion and micro-dilution methods as recommended by NCCLS. The 70% aqueous methanolic extract showed potent activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Total phenol content of the plant showed a substantial amount of phenols (0.97%); in addition the flavonoid rutin was identified by HPLC and MS. The present investigation is the first biological report in fern species *P. vittata* ascertaining the antimicrobial activity; the antimicrobial activity of rutin against the above microorganisms has also been shown.

KEY WORDS.—*Pteris vittata*, rutin, GI microflora, Antimicrobial Agent

The phyotchemical potential of pteridophytes is relatively unexplored, although pteridophytes possess great economic potential due to some interesting medicinal and antimicrobial properties (Chen *et al.*, 2005; Dhiman, 1998; Gogoi, 2002; Reddy *et al.*, 2001; Singh *et al.*, 2001; Singh *et al.*, 2008; Vasudeva, 1999). *Pteris vittata* L., a common fern known as 'Brake Fern', is found all over the world, including India, and its young fronds are used traditionally as an astringent. Its decoction is reported to be used in dysentery and the rhizome is eaten as a tonic after boiling in water (Anonymous, 1969). The species has not been studied thoroughly for its pharmacological properties. Several studies have reported the presence of leucocyanidin, leucodelphinidin, the flavone ester apigenin 7-O-*p*-hydroxybenzoate and a number of glycosides of apigenin, leutolin, isocutellarein-8-*O*-methyl-ether, kaempherol and quercetin (Salantina and Prado, 1998; Imperato, 2006); in addition it has been shown that *P. vittata* hyperaccumulates arsenic (Ma *et al.*, 2001).

In the present study, antimicrobial activity of *P. vittata*, especially against gastrointestinal (GI) pathogens was undertaken. Phytochemical screening of the species was also carried out, and since the presence of flavonoids has been

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reported (Imperato and Telesca, 2000), HPLC analysis was done to find out the presence of some common phenols.

Materials and Methods

Plant material and extraction.—The plants of *P. vittata* were collected from the Fern house of National Botanical Research Institute (NBRI), Lucknow, India in March, 2007. The species was authenticated and the voucher specimens were prepared and deposited in the herbarium of NBRI, Lucknow. Fronds were cut and air-dried at room temperature and coarsely powdered. The powdered material (50 g) was extracted with 70, 80, and 90% aqueous methanol and filtered. The extracts were concentrated under reduced pressure and lyophilised (Labconco, USA) to get dry residue (12.48% w/w, 14.96% w/w, and 19.84% w/w, respectively).

Qualitative phytochemical screening.—Qualitative phytochemical screening of various extracts of the plant material was carried out for qualitative determination of the groups of secondary metabolites present in the material. The methods were followed after Harborne (1973).

Estimation of total phenols.—The total phenolic content of the methanolic extracts was determined spectrophotometrically using Folin-Ciocalteu reagent with gallic acid as the standard (Slinkard and Singleton, 1977). Absorbance measured at 760 nm and gallic acid solutions (10–1000 µg/ml) were used for calibration.

HPLC analysis.—Analyses were performed in a liquid chromatograph with Waters (Milford, MA, USA) pumps equipped with an online degaser, a PCM, Rheodyne 7725 injection valve furnished with a 20 µl loop, a 2996 photodiode array detector and Waters Empower software. Separation was carried out using a Merck Purospher star® (250 × 4.6 mm, i.d., 5 µm pore size) C18 column with guard column of same chemistry. HPLC finger print profile of the phenolics present in the plant was recorded using the reported method for phenolics with slight modifications (Govindarajan *et al.*, 2007). Elution was carried out at a flow rate of 0.8 ml/min with water: phosphoric acid (99.7:0.3 v/v) as solvent A and acetonitrile: water: phosphoric acid (79.7:20:0.3 v/v) as solvent B using a gradient elution in 0–5 min. with 88–85% A, 5–6 min. with 85–82% of A, 6–9.5 min. with 82–75% of A, 9.5–10.5 min. with 75–74% of A, 10.5–12 min. with 74–73% A, 12–20 min. with 73–70% A. Detection was carried out at 264 nm.

Antimicrobial activity assays.—Two different methods were employed for the determination of antimicrobial activities: disc diffusion and microdilution assays as recommended by the National Committee for clinical Laboratory Standards (NCCLS 1999, 2000). All tests were performed in duplicate.

Microbial strains.—*Bacillus cereus* (MTCC, 430), *Escherichia coli* (MTCC, 443), *Klebsiella pneumoniae* (MTCC, 109), *Pseudomonas aeruginosa* (MTCC, 424), *Staphylococcus aureus* (MTCC,96), *Salmonella typhimurium* (MTCC, 98), *Streptococcus pyogenes* (MTCC, 1927), and *Shigella flexneri* (MTCC, 1457) were used as test microorganisms. These test organisms were grown on

Muller Hinton broth (MHB, Oxoid Ltd., Basingstoke, Hampshire, UK) at 37°C for 6–12 h .

Inocula preparation.—Stock bacterial inoculum suspensions were obtained from 6–12 hrs culture on Muller – Hinton broth (MHB,) at 37°C. Those final suspensions served for the inocula preparation. The cell density of each suspensions was determined by NCCLS guidelines (NCCLS 2000) using a counting chamber, adjusted to 0.5 McForland turbidity at the concentration of 10^5–10^6 CFU (Colony forming units)/ml by dilution with MHB.

Antimicrobial assay by Disc–Diffusion assay.—The dried extracts were dissolved in (2.5%) DMSO to a final concentration of 30 mg/ml and sterilized by filtration through 0.45 µm milipore filters. The disc (6 mm in diameter-Himedia) was impregnated with 10 µl of 30 mg/ml crude placed on seeded agar. Erythromycin (30 µg/disc) was used as a positive control and test plates were incubated at 37°C for 18–24 hrs depending on incubation time required for visible growth. Antimicrobial activity was evaluated by measuring the zone of inhibition against organisms.

Standard Microdilution Method (NCCLS).—Tests are performed in sterile U bottom 96- well plates by dispensing into each well 95 µl of MH broth and 5 µl of inoculums (0.5 McForland Tub); 100 µl of test materials were finally added to each appropriate well. The final volume in each well was 200 µl. A standard antibiotic, erythromycin (Sigma), was used as a positive control. The plates were covered with sterile sealer and incubated at 37°C for 18–24 hrs. To indicate bacterial growth 40 µl of 0.2 mg/ml *p*-iodonitroterazodium violet (INT) Sigma solution was added to each well and incubated for another 30 minutes. Inhibition of bacterial growth was visible as a clear well and the presence of growth detected by the presence of pink-red color.

MIC and MBC values.—Inhibition of bacterial growth was visible as a clear well and the presence of growth was detected by the presence of pink- red color. The minimum inhibitory concentration (MICs) values of extract/ compound and the minimum bactericidal concentration (MBCs) of extract, compound and standard drugs were determined by streaking a loopful /5 µl sample of each well (well showing minimum turbidity) onto an over-dried agar (MH) plates and then incubating 37°C for 18–24 h.

Results and Discussion

The antimicrobial activity of three different extracts (70, 80, and 90% aqueous methanol) of *P. vittata*, at a concentration of 30 mg/ml was determined by the diffusion method as shown in Table 1. A total of eight bacterial flora of the gastrointestinal (GI) tract were tested. The percent values of different fractions of *P. vittata* extracts in comparison to a standard antibiotic (erythromycin) were calculated (Table 1). The solvents used for extraction were also used for dissolving the extracts. The 70% extract exhibited a potent activity against all the tested bacteria while the 80 and 90% extracts showed moderate to weak activity against all intestinal pathogens, except *B. cereus*. The solvent controls did not show any activity

TABLE 1. Growth Inhibitory responses (%) of different aqueous methanolic extracts of *P. vittata* against pathogenic intestinal bacteria when compared with erythromicin (30 µg/disc).

Bacterial strains	90%	80%	70%
Escherichia coli 443	65.62	72.29	79.82
Staphylococcus aureus 2672	54.00	68.11	83.42
Bacillus cereus 430	62.58	49.28	54.38
Shigella flexneri 1456	38.92	58.43	62.28
Salmonella typhimurium 98	49.95	67.49	78.01
Streptococcus pyrogenes 1927	59.62	49.38	90.00
Pseudomonas aeruginosa 429	43.48	41.72	121.91
Klebsiela pneumoniae 109	52.62	58.02	102.22

(data not shown) (Table 1). The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) values were also studied for the 70% extract and it showed activity (Table 2). Very low MIC values (0.15 mg/ml) were observed against *P. aeruginosa* and *K. pneumoniae*.

Phytochemical screening showed the presence of flavonoids, tannins, resins, glycosides and terpenoids (Table 3). Total phenol content was also estimated and was found to be 0.97%. Further, HPLC was carried out for the identification and quantification of phenols, and the already reported presence of rutin (Imperato and Telesca, 2000) was confirmed. Identification was based on RT (Figure 1) and spectral match at the start, middle and end of the peak and later by spiking; in addition the presence of rutin was confirmed by MS since the spectrum showed a quasimolecular ion at m/z 609 and a fragment ion at m/z 325 $(quercetin+Na)^+$. The antimicrobial activity of rutin was also tested against all pathogenic bacterial flora of the GI tract and compared with erythromycin to ascertain whether the active principle involved is rutin or some other component. The studies showed that rutin exhibited potent activity against *B. cereus*, *P. aeruginosa* and *K. pneumoniae* with the MIC values of 0.03 mg/ml.

This report shows that *P. vittata* contains potent antimicrobial agents especially against GI pathogens, and the presence of rutin may in part be

TABLE 2. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) values of *P. vittata* 70% extract and compounds against GI pathogens (conc. mg/ml).

	70% aq methanol		Rutin		Erythromycin	
Bacterial strains	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli 443	0.30	0.60	0.07	0.15	0.01	0.03
Staphylococcus aureus 2672	0.30	0.30	0.07	0.31	0.03	0.06
Bacillus cereus 430	1.20	2.40	0.03	0.07	0.01	0.03
Shigella flexneri 1456	0.60	1.20	0.07	0.15	0.03	0.06
Salmonella typhimurium 98	1.20	1.20	0.15	0.31	0.06	0.06
Streptococcus pyrogenes 1927	0.30	0.60	0.07	0.15	0.03	0.12
Pseudomonas aeruginosa 429	0.15	0.30	0.03	0.07	0.01	0.03
Klebsiela pneumoniae 109	0.15	0.60	0.03	0.07	0.01	0.06

TABLE 3. Phytochemical properties of *P. vittata* extract including total phenolic content.

Flavonoids	+
Saponins	−
Tannins	+
Alkaloids	−
Reducing Sugars	−
Resins	+
Glycosides	+
Triterpenoids	+
Steroids	−
Total phenolic content	0.97[a]
Yield 90 : 10	19.84[a]
80 : 20	24.96[a]
70 : 30	20.48[a]

[a]represents % w/w

responsible for added activity. Thus, this report validates the ethnobotanical claims about the species and also warrants more detailed phytochemical and pharmacological investigations in other *Pteris* species, as well as other ferns, which may also be potent sources of antimicrobial agents.

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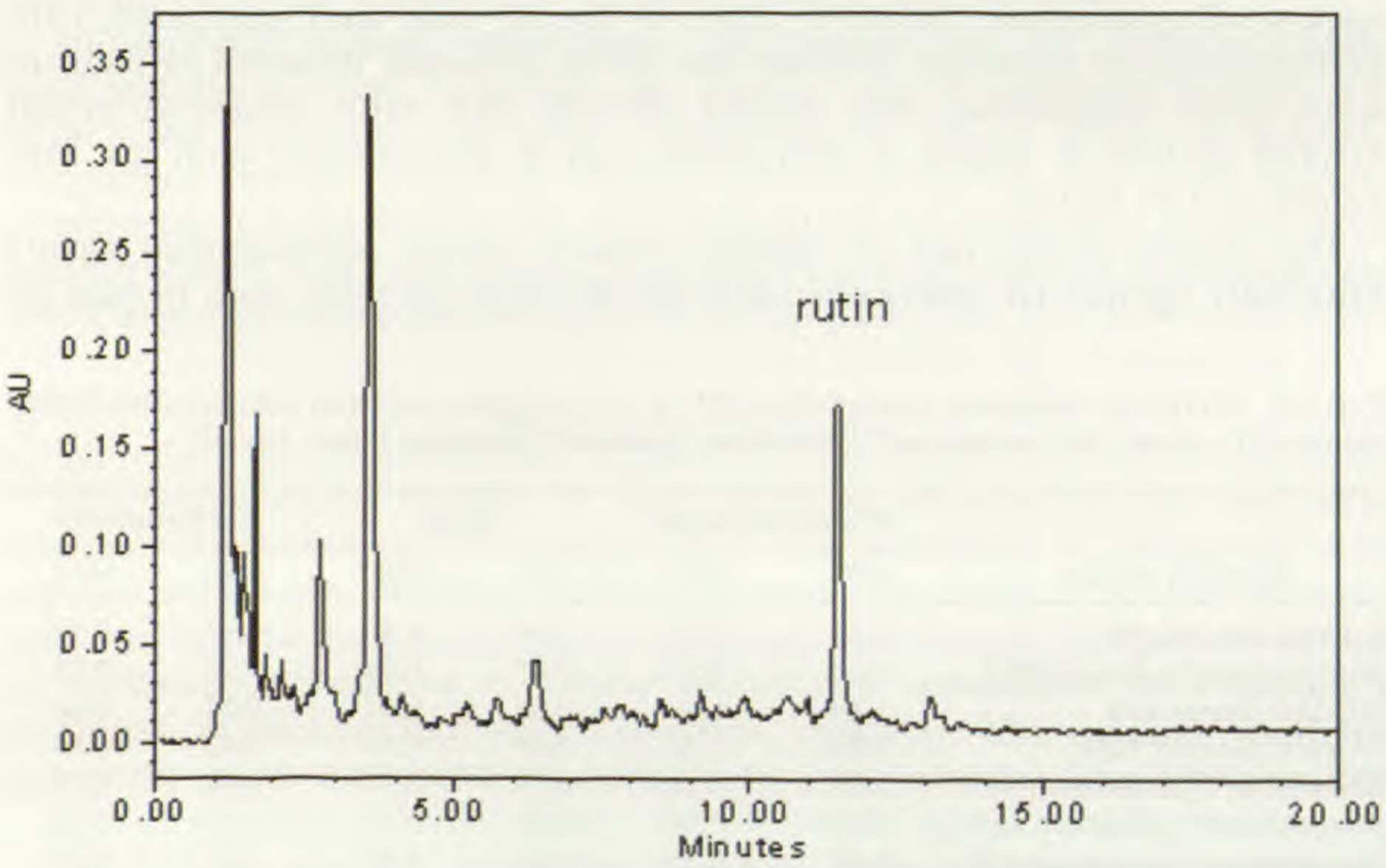

FIG. 1. HPLC finger print profile of phenolics of *P. vittata*.

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